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MAJOR ARTICLE

Clinical and Genomic Characterization of Recurrent Enterococcal Bloodstream Infection in Patients With Acute Leukemia

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Background. Rates and risk factors for recurrent enterococcal bloodstream infection (R-EBSI) and whether the same genetic lineage causes index EBSI and R-EBSI are unknown in patients with acute leukemia (AL) receiving chemotherapy.

Methods. Ninety-two AL patients with EBSI from 2010 to 2015 were included. Enterococcal bloodstream infection was defined by 31 positive blood cultures for *Enterococcus faecium* or *Enterococcus faecalis* and fever, hypotension, or chills. Clearance was defined by 31 negative cultures 324 hours after last positive culture and defervescence. Recurrent enterococcal bloodstream infection was defined by a positive blood culture for *Enterococcus* 324 hours after clearance. Categorical variables were reported as proportions and compared by the χ^2 test. Continuous variables were summarized by median and interquartile range (IQR) and compared by the Wilcoxon-Mann-Whitney Test. *P* values <.05 were considered significant. Whole-genome sequencing was performed on available paired BSI isolates from 7 patients.

Results. Twenty-four patients (26%) had 31 episodes of R-EBSI. Median time to R-EBSI (IQR) was 26 (13–50) days. Patients with R-EBSI had significantly longer durations of fever and metronidazole exposure during their index EBSI. Thirty-nine percent of *E. faecium* R-EBSI isolates became daptomycin-nonsusceptible *Enterococcus* (DNSE) following daptomycin therapy for index EBSI. Whole-genome sequencing analysis confirmed high probability of genetic relatedness of index EBSI and R-EBSI isolates for 4/7 patients.

Conclusions. Recurrent enterococcal bloodstream infection and DNSE are common in patients with AL and tend to occur within the first 30 days of index EBSI. Duration of fever and metronidazole exposure may be useful in determining risk for R-EBSI. Whole-genome sequencing analysis demonstrates that the same strain causes both EBSI and R-EBSI in some patients.

Keywords. acute leukemia; daptomycin-nonsusceptible *Enterococcus*; *Enterococcus*; recurrent bloodstream infection; whole-genome sequencing.

Enterococcal bloodstream infection (EBSI) occurs commonly in patients with acute leukemia receiving chemotherapy at rates of 10.6%–13.9% [1, 2]. However, rates of recurrent enterococcal BSI (R-EBSI) in this patient population are unknown. In addition, whether the same enterococcal genetic lineage causes the index EBSI and R-EBSI in this patient population is unknown. Daptomycin-nonsusceptible *Enterococcus faecium* (DNSE*f*) infection has emerged as a threat to this patient population with limited antimicrobial treatment options. In a single-center study of 77 adult patients with acute leukemia and *E. faecium* BSI, 16 (21%) of patients had DNSE*f* BSI, but rates of DNSE*f* in recurrent infection were not reported [3].

Previous studies have identified risk factors for EBSI, mostly due to *E. faecium*, such as neutropenia, diarrhea, and antibiotic administration including vancomycin, metronidazole, and carbapenems [1, 4, 5]. In theory, these risks persist after the index EBSI as surviving patients will receive more chemotherapy, leading to more neutropenia and antibiotic exposure.

The primary objective of this study is to identify risk factors for R-EBSI in patients with acute myelogenous leukemia (AML) or acute lymphoblastic leukemia (ALL). The secondary objective is to determine whether the same enterococcal genetic lineage causes the index EBSI and R-EBSI using whole-genome sequencing, which offers single-nucleotide resolution and is less prone to producing false-positive or false-negative results [6]. We hypothesize that R-EBSI is common in patients with acute leukemia as the risk for reinfection or relapsed infection persists with immunosuppression from ongoing chemotherapy, prompting further antimicrobial therapy and gut dysbiosis.

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METHODS

Patient Population

This was a retrospective cohort study of 92 patients receiving intensive chemotherapy for AML or ALL admitted to Duke University Medical Center (DUMC) with EBSI from 2010 to 2015. Patients undergoing hematopoietic stem cell transplantation (HSCT) were excluded. Patients were identified as having a positive blood culture for *E. faecium* or *Enterococcus faecalis* in the DUMC Clinical Microbiology Laboratory (CML) during the study period. This study was approved by the DUMC Institutional Review Board.

Definitions

Intensive chemotherapy included induction and consolidation chemotherapy regimens for AML and induction and maintenance therapies for ALL. Bloodstream infection was defined by US Centers for Disease Control and Prevention (CDC) criteria as mucosal barrier injury laboratory-confirmed BSI (MBI-LCBI), central line–associated BSI (CLABSI), or unknown [7].

Enterococcal bloodstream infection was defined by 31 positive blood cultures in the DUMC CML for *E. faecium* or *E. faecalis* and fever, hypotension, or chills. Bloodstream infection clearance was defined by 31 negative culture 324 hours after the last positive culture and defervescence. Fever duration was defined by the number of consecutive days with a temperature 338°C at the time of EBSI. Recurrent enterococcal BSI was defined by a positive blood culture for *E. faecium or E. faecalis* 324 hours after BSI clearance. The definition of R-EBSI was derived from the definition of recurrent *Staphylococcus aureus* BSI [8]. Treatment antibiotics were antibiotics administered empirically or for treatment of another infection whose start date was within 1 week before EBSI.

Antimicrobial exposure was defined by receipt of any antimicrobial within 1 week of the index EBSI. These data and the duration of antimicrobials were obtained through manual chart review. Appropriate enterococcal therapy was receipt of an antibiotic with in vitro activity against the EBSI isolate, as determined by the DUMC CML. Minimum inhibitory concentrations (MIC) were determined by Microscan Gram Positive Panel Type 29 (Baxter Diagnostics, Inc., MicroScan, Sacramento, CA). If vancomycin or daptomycin MICs were not susceptible by the Clinical and Laboratory Standards Institute guidelines, then MICs were confirmed by Etest (bioMérieux, Durham, NC).

Enterococcal Bloodstream Isolates

Paired bloodstream isolates available for 7 patients with R-EBSI, including the index and R-EBSI isolates, were retrieved from frozen storage from the DUMC CML. Isolates for the remaining 17 patients were either not saved at the time of index or recurrent BSI or could not be found in frozen storage. For patients who had >1 episode of R-EBSI, only the isolate from the first episode of R-EBSI was sequenced. Per institutional protocol, microbial isolates from patient blood cultures were stored in glycerol stock at −80°C. Isolates were then plated on trypticase soy agar plates with 5% sheep blood and incubated for 24 hours at 37°C with 5% CO_2. A lawn of bacterial growth was collected using a cotton-tipped swab, resuspended into lysogeny broth, and spun down in a centrifuge to form a cell pellet.

Whole-Genome Sequencing

Genomic DNA for each sample was extracted using the Agencourt Genfind v2 kit. gDNA was then quantified using fluorometric quantitation on a Qubit 3.0 fluorometer (Life Technologies, Carlsbad, CA) using the dsDNA Broad Range Qubit assay. For each sample, 500 ng of gDNA was then sheared using a Covaris S220 ultrasonicator to produce approximately 300-bp DNA fragments, following Covaris' protocol. The sheared DNA was then used to make DNA-Seq libraries using the Kapa BioSystem HyperPrep Library Kit (FisherScientific) following the manufacturer's protocol. Sheared DNA fragments were end-repaired, followed by A-tailing. Illumina standard adapters with unique indexes were ligated, and the library was then amplified by polymerase chain reaction. Resulting libraries were purified using AmPure beads (Beckman Coulter) and quantified using fluorometric quantitation on a Qubit 3.0 fluorometer. The fragment size distribution of each library was checked using an Agilent 2100 Bioanalzyer (Agilent, Santa Clara, CA). Libraries were pooled into equimolar concentration and loaded on an Illumina MiSeq instrument (24 samples per lane for a sequencing depth of 50× coverage). Illumina sequencing was performed using the MiSeq V2 chemistry, and paired-end reads 150 bp in length were generated. DNA extraction, library preparation, and sequencing were performed at the Sequencing and Genomic Technologies Core Facility of the Duke University Center for Genomic and Computational Biology.

Statistical and Bioinformatics Analyses

R, version 3.3.2, and R studio, version 1.0.44, were used for the statistical analysis [9]. Comparisons were made between characteristics of the index EBSI between patients who did not have R-EBSI and those who did have R-EBSI. Categorical variables were reported as proportions and compared by the χ^2 test. Continuous variables were summarized by the median and interquartile range (IQR) and compared by the Wilcoxon-Mann-Whitney Test. A P value of <.05 was considered statistically significant.

Genomes were assembled using SPAdes Genome Assembler [10], and gene prediction was performed using Prodigal Gene Prediction Software [11]. Genome-wide comparison and identification of shared regions were obtained by aligning predicted genes of a genome (the smallest genome assembly) against the rest of the genomes using the BLASTN program [12]. Subsequently, grammar-based genomic distances between the shared genomic regions were computed and applied to

generate the phylogenetic tree using the neighbor-joining method [13, 14]. The phylogenetic tree was annotated using iTOL, version 3 [15]. Pilon was used to determine single nucleotide polymorphisms (SNPs) within the isolates [16]. Whole-genome sequencing data were used to generate the SNP profiles of the paired BSI samples against the reference genomes (*E. faecium* strain ISMMS_VRE_9: NZ_CP018830 and *E. faecalis* strain Symbioflor 1: HF558530). Genomes from the 20 randomly selected *E. faecium* and *E. faecalis* genomes from NCBI (Supplementary Table 1) with the highest number of aligned whole-genome sequencing reads were selected as the reference genomes for the SNP analysis.

RESULTS

Ninety-two patients receiving intensive chemotherapy for AML or ALL developed EBSI during the study period. Twenty-four patients (26%) had 31 episodes of R-EBSI (Table 1). Eighty patients (87%) had index EBSI due to *E. faecium* whereas 12 patients (13%) had index BSI due to *E. faecalis* (Table 2). There were no significant differences between antibiotic susceptibility patterns of the index enterococcal BSI isolates between patients without R-EBSI and patients with R-EBSI.

Among patients with R-EBSI, the median time from index EBSI to R-EBSI (IQR) was 26 (13–50) days, and *E. faecium* was the most common infecting pathogen (23/24; 96%). Four patients with index EBSI due to *E. faecalis* during their index infection had R-EBSI due to *E. faecium*. Among the patients with R-EBSI due to *E. faecium*, 9 (39%) had BSI isolates that converted from daptomycin-susceptible to DNSE*f*. Three patients with index EBSI due to vancomycin-resistant *E. faecium* (VRE) had R-EBSI due to vancomycin-susceptible *E. faecium* (paired isolates were not available in storage for sequencing). Of the 24 patients with R-EBSI, 4 (17%) had >1 episode of R-EBSI. One patient with 3 episodes of R-EBSI had DNSE*f* BSI during the third recurrence following daptomycin therapy for the second recurrence.

There were no significant differences between time from leukemia diagnosis to EBSI, history of relapsed or refractory

Table 1. Baseline Demographics of Index Enterococcal Bloodstream Infection

Characteristic	No Recurrent Enterococcal BSI (n = 68; 74%)	Recurrent Enterococcal BSI (n = 24; 26%)	*P* Value
Female, No. (%)	25 (37)	10 (42)	.48
Age, median (IQR), y	62 (51–70)	61 (46–67)	.58
Race, No. (%)			
White	54 (79)	18 (75)	.30
Black	11 (16)	4 (17)	.99
Other	3 (5)	2 (8)	
Ethnicity, No. (%)			
Hispanic	1 (2)	0	
Non-Hispanic	23 (98)	24 (100)	
Underlying disease, No. (%)			
AML	59 (87)	20 (83)	.68
ALL	9 (13)	4 (17)	
Prior hematologic malignancy, No. (%)	16 (24)	9 (38)	.19
Time from leukemia diagnosis to first BSI, median (IQR), d	30 (19–108)	30 (18–164)	.97
First induction chemotherapy regimen			
7 + 3	52 (77)	18 (75)	.89
CALGB	7 (10)	2 (8)	
Other	9 (13)	4 (17)	
Relapsed/refractory disease at time of BSI	36 (53)	15 (63)	.42
Timing of BSI in relationship to chemotherapy, No. (%)			
Induction 1	33 (49)	10 (42)	.56
Induction 2	20 (29)	8 (33)	.72
Induction 3	9 (13)	4 (17)	
Induction 4	2 (3)	2 (8)	
Induction 5	2 (3)	0	
Consolidation, No. (%)	2 (3)	0	
Prior BSI not due to *Enterococcus* (since acute leukemia diagnosis), No. (%)	32 (47)	14 (58)	.34
Total treatment antibiotic days, median (IQR)			
Cefepime	15 (9–22)	22 (12–32)	.09
Vancomycin (IV)	8 (5–16)	8 (4–22)	.59
Imipenem/meropenem	19 (14–29)	17 (10–22)	.80
Metronidazole	11 (8–14)	15 (15–17)	**.03**

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BSI, bloodstream infection; IQR, interquartile range; IV, intravenous.

Table 2. Index Enterococcal Bloodstream Infection Characteristics

Characteristic	No Recurrent Enterococcal BSI (n = 68; 74%)	Recurrent Enterococcal BSI (n = 24; 26%)	*P* Value
Length of stay before BSI, median (IQR), d	17 (14–27)	17 (15–29)	.58
Diarrhea at time of BSI, No. (%)	28 (41)	13 (54)	.27
Diarrhea due to *Clostridium difficile*, No. (%)	14 (21)	4 (17)	.68
Mucositis at time of BSI, No. (%)	19 (28)	3 (13)	.13
Duration of fever with BSI, median (IQR), d	2.5 (1–5)	6 (2–7)	**.03**
Duration of neutropenia before BSI, median (IQR), d	12 (7–16)	10 (7–19)	.38
Total duration of neutropenia at time of BSI, median (IQR), d	21 (17–29)	23 (19–76)	.86
Time to BSI clearance, median (IQR), d	3 (1–4)	3 (2–5)	.11
Enterococcus species, No. (%)			
E. faecium	61 (90)	19 (79)	.19
E. faecalis	7 (10)	5 (21)	
Antibiotic resistance, No. (%)			
Ampicillin	54 (79)	16 (67)	.2
Vancomycin	49 (72)	14 (58)	.2
Daptomycin	4 (6)	0	
Polymicrobial BSI, No. (%)	15 (22)	4 (17)	.68

Abbreviations: BSI, bloodstream infection; IQR, interquartile range.

leukemia at the time of BSI, or timing of BSI in relationship to chemotherapy cycle. Patients who developed R-EBSI had a significantly longer duration of metronidazole exposure at the time of index EBSI compared with patients without R-EBSI (15 days; IQR, 15–17 days; vs 11 days; IQR, 8–14 days; $P = .03$). Mucosal barrier injury laboratory-confirmed BSI was the most common source for BSI in both groups based on CDC criteria with *Enterococcus* as an intestinal organism and the presence of neutropenia within 7 days of the positive blood culture in 90 out of the 92 patients (98%).

Patients who developed R-EBSI had a significantly longer duration of fever (including time pre- and post-BSI) than patients who did not have R-EBSI (6 days; IQR, 2–7 days; vs 2.5 days; IQR, 1–5 days; $P = .03$) (Table 2). There were no significant differences between ampicillin, vancomycin, or daptomycin resistance of the index EBSI isolate or duration of BSI. Two patients without R-EBSI had secondary sites of enterococcal infection with their index BSI including meningitis and empyema, whereas no patients with R-EBSI had secondary sites of infection during their index EBSI.

There were no significant differences between time to appropriate enterococcal therapies and rates of removal of vascular catheters for the index EBSI (Table 3). Daptomycin was the most commonly prescribed anti-enterococcal therapy in both groups (49 patients in both groups). Fifty-three percent of patients' therapy was switched, most commonly to linezolid (28 out of 49 patients) at the time of discharge.

Whole-genome sequencing confirmed high probability of genetic relatedness of index EBSI and R-EBSI isolates in 3 out of 7 patients, inferring that both isolates are from the same enterococcal genetic lineage (Table 4). Of note, patients 1–6 had index and R-EBSI due to the same species of *Enterococcus* whereas patient 7 had an index EBSI due to *E. faecalis* and R-EBSI due to *E. faecium*. The paired isolates from patients 1, 3, and 5 were predicted to have 99.4%, 99.6%, and 99.1% of shared genes with 100% identity, respectively. An analysis of SNPs supported the finding that patients 1, 3, and 5 have isolates of the same enterococcal genetic lineage with ≤5 different SNPs, indicating a high degree of genetic relatedness. Supplementary Data 1 depicts the shared set of genes between all isolates (the core genomes) that had <95% identity between the index and R-EBSI isolates. We suspect that these SNPs caused the identity to drop below the threshold of 95%.

Figure 1 is a phylogenetic tree placing the sequenced isolates from the 7 patients in this study among the previously characterized *E. faecium* and *E. faecalis* isolates. A close phylogenetic relationship is seen between paired EBSI isolates from patients 1, 3, and 5.

Table 3. Index Enterococcal Bloodstream Infection Outcomes

Characteristic	No Recurrent Enterococcal BSI (n = 68; 74%)	Recurrent Enterococcal BSI (n = 24; 26%)	*P* Value
Vascular catheter removed for BSI, No. (%)	34 (50)	11 (46)	.73
Time to appropriate antibiotics, median (IQR), d	2 (1–3)	3 (2–3)	.23
Appropriate enterococcal antibiotic, No. (%)			
Vancomycin	8 (12)	5 (21)	.45
Daptomycin	54 (79)	18 (75)	
Other	6 (9)	1 (4)	

Abbreviations: BSI, bloodstream infection; IQR, interquartile range.

Table 4. Whole-Genome Sequencing of Paired Bloodstream Isolates From 7 Patients

Patient	Index BSI Species	Recurrent BSI Species	Days Between BSIs	Central Line Removed for BSI	Total Length of Predicted Genes, Megabase Pairs; Index BSI Isolate	Shared Genes With >95% Identity, %	Shared Genes With 99% Identity, %	Shared Genes With 100% Identity, %	Shared SNP Positions	Different SNP Positions
1	*E. faecium*	*E. faecium*	8	Yes	2 311 662	99.8	99.8	99.4	5522	5
2	*E. faecium*	*E. faecium*	25	Yes	2 364 525	92.7	91.0	82.2	2930	51
3	*E. faecium*	*E. faecium*	15	No	2 311 245	99.8	99.8	99.6	60	1
4	*E. faecalis*	*E. faecalis*	49	No	2 640 342	85.2	58.7	4	6525	102
5	*E. faecium*	*E. faecium*	10	No	2 348 367	99.4	99.4	99.1	76	0
6	*E. faecium*	*E. faecium*	32	No	2 468 892	85.5	83.4	66.8	3655	29
7	*E. faecalis*	*E. faecium*	26	No	2 291 253	0.63	0.60	0.45	N/A	N/A

Abbreviations: BSI, bloodstream infection; SNP, single nucleotide polymorphsim.

DISCUSSION

This is the largest cohort of patients with acute leukemia and R-EBSI reported to date. R-EBSI is common (26%) in patients with AML and ALL receiving intensive chemotherapy and tends to occur within 30 days of the index EBSI. Among the 7 patients for whom paired BSI isolates were available, 43% (3/7) have index and R-EBSI due the same enterococcal genetic lineage. The use of whole-genome sequencing in the present study offers greater resolution in comparing strain-relatedness with traditional techniques such as pulsed-field gel electrophoresis (PFGE) [6].

Prior studies of R-EBSI have provided conflicting conclusions. In a study of 27 patients with 60 episodes of EBSI over 10 years, 8 patients had infection with isolates from the same genetic lineage based on multilocus sequence typing. Durations between BSI episodes ranged from 3 months to 6 years [17]. In a study of R-EBSI due to VRE BSI, investigators identified 3 out of 36 patients having R-EBSI due to VRE (8.3%) and analyzed these isolates for genetic relatedness using PFGE [18].

In a study analyzing 26 R-EBSI isolates from 12 patients using whole-genome sequencing, investigators found that 10 (71%) R-EBSI episodes were due to relapsed infection with a different genetic lineage [19]. Notably, the definition of R-EBSI in this study only included recurrence ≥30 days after the index BSI. Based on our findings, we assert that any recurrence, even if only 1–2 weeks after the index EBSI, is clinically relevant due to the high risk for DNSE*f*, potential for subsequent central line removal, and delays in chemotherapy.

In the present study, traditional clinical factors associated with risk for recurrent BSI, such as length of hospital stay, persistent BSI, delay in appropriate antibiotic therapy, failure to remove the central venous catheter, and secondary sites of infection, were not associated with R-EBSI. As most of the cases of EBSI were MBI-LCBI, and DNSE*f* BSI was more common in R-EBSI following daptomycin exposure, we suspect that the risk for R-EBSI is more complicated than what could be explained by a single clinical risk factor. Theoretically, mucosal barrier injury from cytotoxic chemotherapy leads to the index EBSI, which remains a constant risk while patients are still neutropenic. In therapy for acute leukemia, once patients recover their neutrophil counts, they often go on to receive more chemotherapy, re-introducing the risk for MBI-LCBI. In addition, treatment with daptomycin leads to selective pressure on the gut microbiota for emergence of DNSE*f* and potential for recurrent infection.

Longer fever duration was also a significant risk factor associated with R-EBSI. In a previous study of 909 episodes of febrile neutropenia and BSI in cancer patients, the investigators found that patients with complex BSI including a secondary site of infection such as pneumonia or soft tissue infection had significantly longer durations of fever compared with patients with simple BSI (and no secondary site of infection) [20]. Patients with complex BSI were also less likely to achieve a clinical response to initial antimicrobial therapy.

In the present study, although no patient with R-EBSI had known secondary sites of infection, fever duration could correlate with a more severe index EBSI with a higher likelihood of failing initial anti-enterococcal therapy. However, it was surprising that fever duration was significantly longer among patients who went on to have R-EBSI, without a significant difference in time to BSI clearance.

Longer metronidazole exposure was also a risk factor for R-EBSI. A previous study demonstrated that depletion of the commensal anaerobic gut microbiota in allogeneic HSCT recipients led to reduced gut microbiota diversity and increased risk of mortality [21]. In another study, investigators found that metronidazole was a risk factor for reduced microbiota diversity. Metronidazole was also associated with increased risk for intestinal domination due to *Enterococcus*, where *Enterococcus* accounted for ≥30% of the gut microbiota [22]. In turn, patients with enterococcal intestinal domination had a 9-fold increased risk for subsequent VRE BSI.

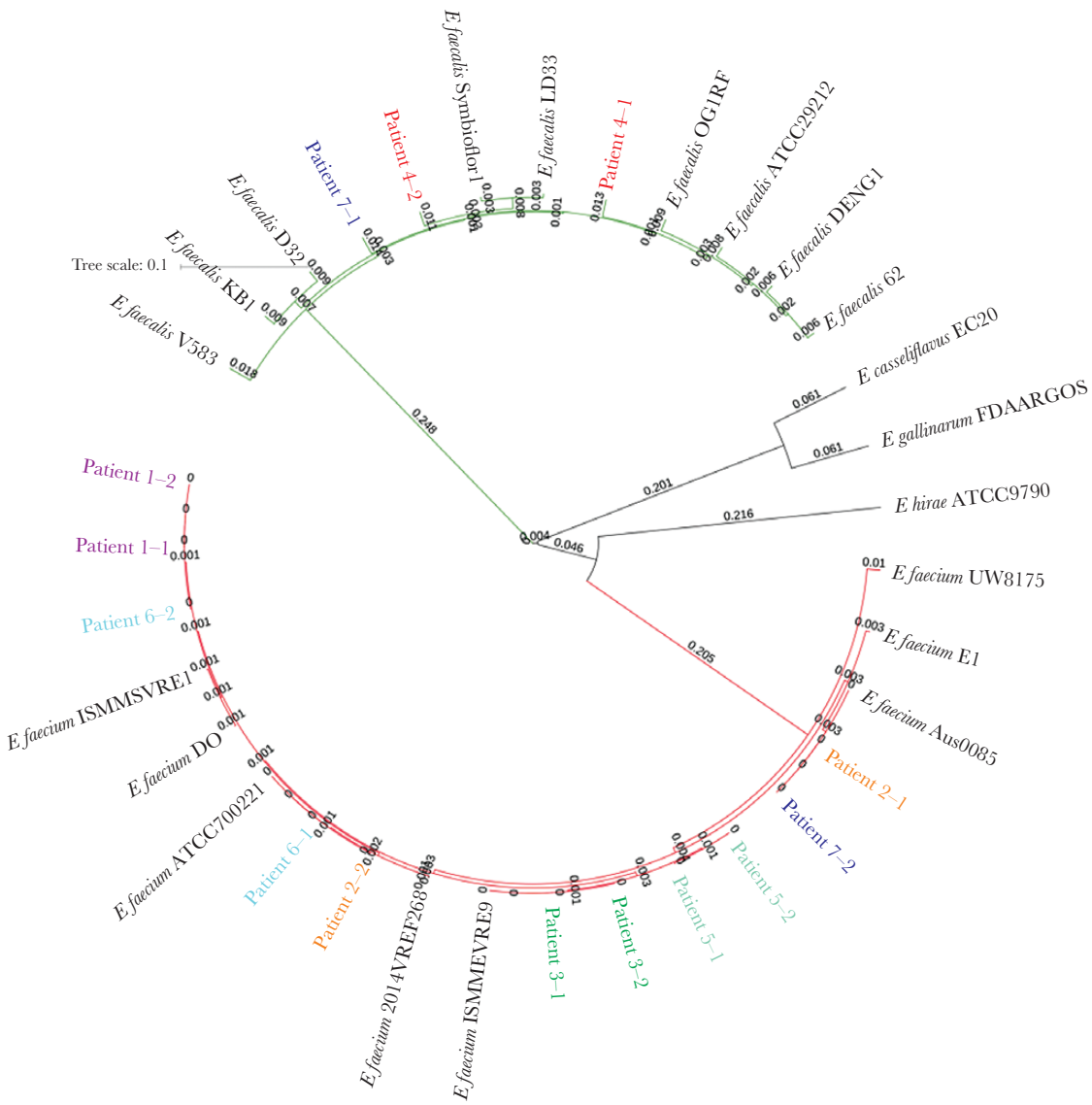

Figure 1. Phylogenetic analysis of paired *Enterococcus* isolates from 7 patients with recurrent enterococcal bloodstream infection in relationship to previously characterized isolates. Patient number 1 represents the enterococcal bloodstream isolate from the index enterococcal bloodstream infection, and patient number 2 represents the enterococcal bloodstream isolate from the recurrent infection. Paired isolates from each patient are color-coded, with the 2 isolates from the same patient being of the same color. Reference strain citations are in Supplementary Table 1.

In the present study, R-EBSI due to DNSE*f* (39%) was notable and likely due to selective antimicrobial pressure on the gut microbiota from preceding daptomycin therapy. In a study of adult acute leukemia patients, DiPippo et al. found that receiving daptomycin for ≥13 days within 90 days of BSI significantly correlated with the development of DNSE*f* infection [3]. Based on findings from the present study, we propose that R-EBSI in addition to prior daptomycin exposure should be taken to account in assessing risk for DNSE*f*.

Of note, 4 patients in the present study with index DNSE*f* BSI did not go on to have R-EBSI. Additionally, none of the 4 patients with DNSE*f* BSI during their index EBSI had any known prior daptomycin exposure, which prompts the need for further investigation into whether the DNSE*f* isolates were hospital-derived isolates that the patients acquired through environmental contamination or part of the patients' commensal microbiota at the start of chemotherapy.

Among the 6 patients with available sequencing data and the same *Enterococcus* species causing index and R-EBSI, 3 patients had index and R-EBSI due to the same strain of *Enterococcus*, and 3 patients had index and R-EBSI due to different genetic lineages of *Enterococcus*. Among the 3 patients with the same infecting genetic lineages, the duration between the index and R-EBSI ranged from 8–15 days whereas the duration for the other 3 patients' index and R-EBSI ranged from 25 to 49 days. This finding correlates with a previous study that isolates are more likely to be of the same genetic lineage when there is a shorter duration between the index and R-EBSI [18].

The present study cannot make conclusions as to whether central venous catheters should be removed to prevent R-EBSI due to a limited sample size. However, 2 patients who underwent catheter removal for their index EBSI still had the same strain of *Enterococcus* causing R-EBSI, suggesting an alternative source of R-EBSI such as the gastrointestinal tract. In a prior study of 111 adult patients with enterococcal CLABSI, the central venous catheter was retained in 29.1% of patients, and this was not associated with R-EBSI [23]. However, catheter retention was significantly associated with a higher in-hospital mortality rate (37.9% vs 18.3%) following index EBSI. The authors suggested that the higher in-hospital mortality rate may have been due to patients being too ill for catheter removal to be performed safely or having poor vascular access. Regardless, coupled with our findings, catheter retention may not lead to R-EBSI, but further investigation is needed into additional negative outcomes.

Limitations of the present study include the single-center retrospective design and sequencing of paired isolates from only 7 out of 24 patients with R-EBSI. Other centers appear to have lower rates of EBSI (10.6%–13.9%) in the acute leukemic population compared with DUMC (27%) [24], in addition to possible varying practices with antimicrobial prophylaxis and febrile neutropenia protocols. Despite *E. faecalis* accounting for 13% of the index EBSIs in this study, only 1 patient went on to have R-EBSI due to *E. faecalis*, and this limits our ability to generalize the findings of the clinical and genomic analyses to both *E. faecalis* and *E. faecium* infections. For future studies, a larger sample size of patients with R-EBSI will be needed to investigate the reproducibility of the metronidazole exposure and fever duration as risk factors. Additionally, a larger sample size of paired bloodstream isolates to investigate the rate of genetic lineage relatedness will be helpful in determining whether R-EBSI is due to a re-infection with the same genetic lineage or relapsed infection with a different genetic lineage.

In conclusion, fever duration may identify patients at risk for R-EBSI, and reducing metronidazole exposure as an antimicrobial stewardship strategy may prevent R-EBSI. As most patients with R-EBSI have a gut-derived source of infection, future studies of how gut dysbiosis occurs and how the gut microbiota repopulates over the course of chemotherapy will be helpful in determining which patients are at risk for R-EBSI. In addition, a larger sample size of paired EBSI isolates is needed to draw conclusions on how frequent R-EBSI episodes are due to the same genetic lineage as index episodes and whether this finding is related to central venous catheter retention.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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